Covalent stabilization of coiled coils of the HIV gp41 N region yields extremely potent and broad inhibitors of viral infection

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Peptides from the N-heptad repeat region of the HIV gp41 protein can inhibit viral fusion, but their potency is limited by a low tendency to form a trimeric coiled-coil. Accordingly, stabilization of N peptides by fusion with the stable coiled-coil IZ yields nanomolar inhibitors [Eckert, D. M. & Kim, P. S. (2001) *Proc. Natl. Acad. Sci. USA* 98, 11187–11192]. Because the antiviral potency of IZN17 is limited by self-association equilibrium, we covalently stabilized the peptide by using interchain disulfide bonds. The resulting covalent trimer, $(CCIZN17)_3$, has an extraordinary thermodynamic stability that translates into unprecedented antiviral potency: $(CCIZN17)_3$ (*i*) inhibits fusion in a cell–cell fusion assay (IC_{50} = 260 pM); (*ii*) is the most potent fusion inhibitor described to date (IC_{50} = 40–380 pM) in a single-cycle infectivity assay against HIV_{HXB2}, HIV_{NL4-3}, and HIV_{MN-1}; (*iii*) efficiently neutralizes acute viral infection in peripheral blood mononuclear cells; and (*iv*) displays a broad antiviral profile, being able to neutralize 100% of a large panel of HIV isolates, including R5, X4, and R5/X4 strains. In all of these assays, the potency of N-peptide inhibitor $(CCIZN17)_3$ was equal to or more than the C-peptide inhibitor in clinical use, DP178 (also known as Enfuvirtide and Fuzeon). More importantly, we show that the two inhibitors, which have different targets in gp41, synergize when used in combination. These features make $(CCIZN17)_3$ an attractive lead to develop as an antiviral drug, alone or in combination with DP178, as well as a promising immunogen to elicit a fusion-blocking neutralizing antibody response.

fusion inhibitor

HIV type 1 (HIV-1) infects cells by fusing its membrane with the host cell membrane. The key player in viral entry into target cells is the envelope glycoprotein complex gp160, which is composed of the subunits gp120 and gp41. Before exposure to cellular receptors, the two subunits form a noncovalent complex that associates in a trimer to form spikes at the virus surface. Binding of gp120 to the host cellular receptors triggers a cascade of conformational changes in the envelope complex, culminating with the insertion of the transmembrane subunit gp41 into the host cell membrane. The mechanism of fusion involves two helical regions of gp41, an N-terminal heptad repeat (HR), HR1 (defined as N helix or N peptide in various studies), and a C-terminal HR, HR2 (defined as C helix or C peptide). HR1 and HR2 form a fusogenic (i.e., fusion-active) conformation called the "trimer-of-hairpins," a structure common to the fusion mechanism of many enveloped viruses (1, 2) consisting of a bundle of six α-helices contributed by three gp41 monomers. Three C peptides, one per gp41 chain, pack in an antiparallel manner against a central three-stranded coiled-coil formed by the N regions of the same chains (3–6). It is generally accepted that fusion progresses by formation of an intermediate, a "prehairpin" conformation, that places the N-terminal fusion peptide near or in the target cell membrane, exposing the HR1 and HR2 regions (1). In this intermediate, both HR are vulnerable to binding by synthetic C and N peptides, which can thus inhibit viral infection by preventing formation of the fusogenic trimer-of-hairpins. C peptides are potent inhibitors of HIV-1, active at low nanomolar concentration (7, 8). One of them, DP178 or T-20, has been approved with the name Enfuvirtide (also known by its brand name Fuzeon) as the first member of a new class of antiretroviral drugs known as fusion inhibitors (9, 10).

Antiviral activity was also reported for N peptides. Two mechanisms were proposed: interference with trimer-of-hairpin formation by binding to the C peptides (1, 11, 12) or disruption of the inner coiled-coil by intercalating within the N helices (3, 13). Linear N peptides are generally far less potent than C peptides, possibly because in the absence of the latter they aggregate and are sequestered in nonproductive intermolecular assemblies. Accordingly, a correlation exists between the solution structure of N peptides and their antiviral activity (7, 8, 14).

Starting from this observation, very potent engineered peptides were described. Root *et al.* (15) designed the single-chain construct 5-helix, with alternating N and C helices (three and two, respectively) linked by interconnecting loops. 5-helix folds into a structure similar to the trimer-of hairpins, but with an unoccupied binding site for a C peptide. Accordingly, it binds a C peptide very efficiently, and inhibits HIV-1 at low nanomolar concentration. Louis *et al.* (16) designed a soluble trimeric coiled-coil, N_{CCG}-gp41, with the N helix fused in helical phase to a minimal thermostable gp41 trimeric core and the smaller, soluble constructs $N34_{CCG}$ and $N35_{CCG}$-N13, consisting of the internal trimeric gp41 coiled-coil, stabilized by intermolecular disulfide bonds (17). The increased stability translated to nanomolar inhibitory potency. Importantly, one of the constructs, $N35_{CCG}$-N13, was able to elicit neutralizing antibodies (17), thus validating the prehairpin intermediate as a vaccine target.

Other low nanomolar N-peptide inhibitors were reported by Eckert and Kim (11). These inhibitors were chimeric molecules, consisting of a designed trimeric coiled-coil (18, 19) fused to a portion of the N helix. Eckert and Kim showed that the inhibitory activity was correlated to the stability of the coiled-coil structure.

Overall, these studies demonstrated that appropriate engineering of N peptides could increase their inhibitory potency by three orders of magnitude. Building on these observations, we describe here chimeric N peptides that combine the concept of fusion with a stable coiled-coil domain with the concept of covalent stabilization by using disulfide bonds. We show that these covalent chimeric constructs display antiviral potency in the picomolar range, with a remarkable breadth of neutraliza-

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Abbreviations: HIV-1, HIV type 1; HR, heptad repeat; PBMC, peripheral blood mononuclear cell.

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Table 1. Sequences of HIV-1 gp41 N helix and derived chimeric peptides inhibitors

Peptide	Sequence
	d a d a d a d a d a d a d
N helix	QARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLK
N36	SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARIL
N23	IEAQQHLLQLTVWGIKQLQARIL
N17	LLQLTVWGIKQLQARIL
IZN36	*IKKEIEAIKKEQEAIKKKIEAIEKEI*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARIL
IZN23	*IKKEIEAIKKEQEAIKKKIEAIEKEI*EAQQHLLQLTVWGIKQLQARIL
IZN17	*IKKEIEAIKKEQEAIKKKIEAIEK*LLQLTVWGIKQLQARIL
N17IZ	LLQLTVWGIKQLQARIL*AIKKEIEAIKKEQEAIKKKIEAI*
CCIZN17	*CCGGIKKEIEAIKKEQEAIKKKIEAIEK*LLQLTVWGIKQLQARIL
CCIZN23	*CCGGIKKEIEAIKKEQEAIKKKIEAIEKEI*EAQQHLLQLTVWGIKQLQARIL
CCI10N17	*CCGGIKKKIEAIEK*LLQLTVWGIKQLQARIL

The HR1 sequence corresponds to residues 540–588 of HIV-HXB2, with alignment of corresponding positions a and d of the heptad repeats of the coiled-coil. Non-HIV residues are shown in italic; HIV residues are underlined. All peptides feature C-terminal carboxyamide and N-terminal acetyl, except IZN23 and CCIZN23 (which have N-terminal biotin).

tion against a large panel of HIV strains, including many primary isolates.

Materials and Methods

Peptide Synthesis and CD. All of the peptides in Table 1 were synthesized by solid-phase synthesis. For full details on peptide synthesis and CD, see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

HIV-1 Neutralization Assays. Three different assay formats were used. The first format was a single-cycle HIV-1 infectivity assay. P4–2/R5 cells (20, 21) are HeLa cells stably expressing human CD4 and CC-chemokine receptor 5 and harboring a β-galactosidase reporter gene driven by a tat-responsive fragment of the HIV-2 LTR. P4–2/R5 cells were maintained at 37°C and 5% CO_2 in phenol red-free DMEM/10% FBS. For infectivity assays, cells were seeded in 96-well plates (Costar) at 2.5×10^3 cells per well and infected the following day with HIV-1 at a multiplicity of infection of 0.01 in the presence of peptides. Forty-eight hours later, cells were lysed and β-galactosidase activity was measured with a chemiluminescent substrate as described in ref. 22.

The second format was a spread assay using MT4 cells or peripheral blood mononuclear cells (PBMCs). Cells at 4×10^5 per ml (MT4 cells) or 10^6 per ml (PBMCs) were infected at a multiplicity of infection of 0.01, washed free of virus 24 h after infection and seeded into wells of the 96-well plates. Test peptides were diluted by two-fold serial dilutions and mixed with cells. Details of plate assembly for the $(CCIZN17)_3$/DP178 combination experiment are given in *Supporting Materials and Methods*. Cultures were incubated at 37°C and 5% CO_2 for 72 h and then assayed for viral production by a commercial p24 assay kit (Beckman Coulter).

The third format was a commercial phenotypic virus assay (ViroLogic, South San Francisco, CA) described in ref. 23.

HIV-1 Cell-Cell Fusion Assay. Inhibition of cell–cell fusion was measured by using a quantitative assay essentially as described in ref. 24. Briefly, inhibitors were titrated into cultures containing a mixture of SupT1 cells overexpressing cytoplasmic β-lactamase and HeLa cells expressing HXB2 gp160. HeLa cells were also loaded with the β-lactamase fluorescent substrate, CCF4-AM. Fusion was measured by quantifying CCF4 fluorescence in the blue and green channels, with the blue/green fluorescence ratio being proportional to the extent of fusion.

Results

Structure Activity Relationship of Chimeric N-Peptide Inhibitors. Our starting point was the study by Eckert and Kim (11). These authors compared the antiviral potency of chimeric N peptides consisting of the designed trimeric coiled-coil IZ (18, 19) fused to portions of the gp41 N helix, namely IZN17, IZN23, and IZN36 (Table 1). N17 is the portion of the N region comprising the gp41 hydrophobic pocket (6, 25). N23 and N36 include 6 or 19 (respectively) additional residues upstream to the N17 region (Table 1). In addition to providing an important set of contacts for the cognate C peptide during formation of the trimer-of-hairpin (6, 25), the N17 region contributes significantly to the stability of HR1 self-association, which is completely abolished by deletion of the hydrophobic pocket (26). IZN17, IZN23, and IZN36 all have similar inhibitory potency (11), suggesting that the antiviral activity of this class of chimeric N peptides is recapitulated in the N17 portion of the N helix, which we used for all our subsequent work.

To complement the data reported by Eckert and Kim (11), we investigated how the position of the IZ scaffold influences antiviral potency. We prepared the analog N17IZ, with the IZ domain C-terminal instead of N-terminal to the N17 sequence. An alanine was included between N17 and IZ to maintain the alternating *a*-to-*g* heptad repeat in frame (Table 1). Examination of the structure of the fusion-active 6-helical bundle suggests that, although IZN17 should be able to capture the N-terminal portion of the C helix, the C-terminal portion of the C helix would have to partially twist in order not to clash against the coiled-coil groove of IZ. By positioning the IZ domain in a different structural context, one could hope to reduce the steric hindrance to C-peptide binding, hence improving antiviral potency.

N17IZ is fully helical at 10 μM as its parent peptide IZN17 (Table 2; see also Fig. 6, which is published as supporting information on the PNAS web site). N17IZ is also very stable, with a melting temperature (T_m) of >90°C. In the presence of 2 M guanidinium chloride, N17IZ shows a cooperative thermal transition, with T_m = 50.7°C, whereas, for IZN17, T_m = 61.5°C (Table 2 and Fig. 1). Despite the preserved structural stability, N17IZ shows dramatically reduced antiviral activity, with IC_{50} = 800 nM, almost three orders of magnitude lower than IZN17 (Table 2). Structural studies have shown that the N-helix bundle extends only seven more residues at the C terminus of N17, followed by a region interconnecting the N and C helices (3). Our data suggest that a C-terminally positioned IZ domain may clash with some region of the fusion intermediate other than the C helix.

Covalently Trimeric Chimeric N-Peptide Inhibitors. The above data confirmed that the chimeric peptide IZN17 represented the best

Table 2. Biophysical data and HIV-1 inhibitory activity of chimeric N peptides

Peptide	Helicity at 10 μM	T_m, °C	T_m 2 M GdnHCl, °C	IC_{50}, nM
IZN17	99	>90	61.5	1.26
N17IZ	96	>90	50.7	799.94
IZN23	nd	>90	nd	2.33
$(CCIZN17)_3$	97	>90	>90	0.04
$(CCIZN23)_3$	100	>90	>90	0.31
CCI10N17	65	nd	nd	nd
$(CCI10N17)_3$	85	60	nd	23.76
5-helix	nd	nd	nd	9.93
DP178	nd	nd	nd	1.14

Helicity was determined by CD spectroscopy. T_m is the midpoint of thermal denaturation transitions by CD spectroscopy. Shown is T_m determined alone and T_m in the presence of 2 M guanidinium chloride. IC_{50} values were determined from a single-cycle infectivity assay using HIV-HXB2. n.d., not done.

starting point for further improvement. We reasoned that, in this molecule, the presence of a coiled-coil structure depends on the monomer/trimer equilibrium in solution, and, thus, strictly depends on concentration. The observed antiviral potency of IZN17 should therefore result from the interplay between the binding constant of the trimer to the C peptide and the constant of oligomerization to form the coiled-coil. Rather than try to design more stable trimerization scaffolds to overcome this putative limitation, we designed a construct, CCIZN17, which can form a covalently stabilized coil-coil structure (Table 1 and Fig. 2). Similar to Louis *et al.* (16, 17), we introduced two cysteine residues in the monomeric IZN17 precursor to form three interchain disulfide bridges upon spontaneous assembly of the monomers into a trimer. Instead of introducing the cysteines inside the heptad repeat region of the coiled-coil as in the Louis *et al.* design (16, 17), we located a cysteine pair at the N terminus of the IZ domain that was spatially separated from the coiled-coil domain by two additional glycine residues. The glycine residues would endow the pair of cysteines with sufficiently high conformational freedom during the spontaneous formation of the interchain disulfide bonds.

When dissolved at neutral pH, IZN17 folds spontaneously into a stable trimeric coiled-coil, with three peptide chains physically associated in parallel orientation (Fig. 2) (11). Similarly, three identical cysteine-containing molecules of CCIZN17 associate into a trimeric coiled-coil structure, and the resulting close proximity of the juxtaposed cysteine residues allows the formation of three intermolecular disulfide bonds under oxidizing conditions, as shown schematically in Fig. 2 and experimentally with HPLC-MS in Fig. 3.

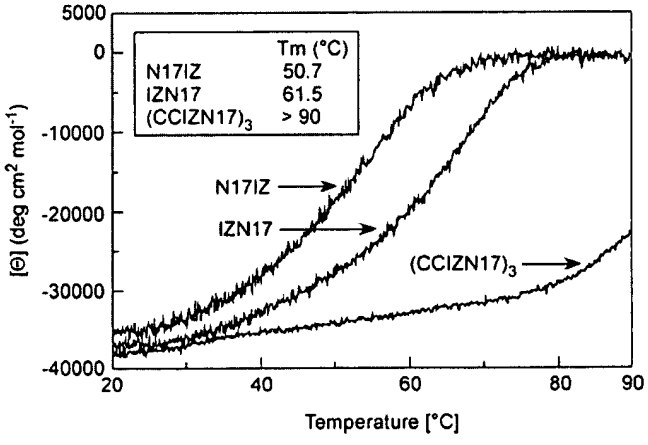

Fig. 1. Thermal denaturation curves of IZN17, N17IZ, and $(CCIZN17)_3$, as monitored by CD at 222 nm in 5 mM Hepes, pH 7.3/150 mM NaCl/2 M guanidinium hydrochloride. The melting temperatures (T_m), are 50.7°C, 61.5°C, and >90°C for N17IZ, IZN17, and $(CCIZN17)_3$, respectively.

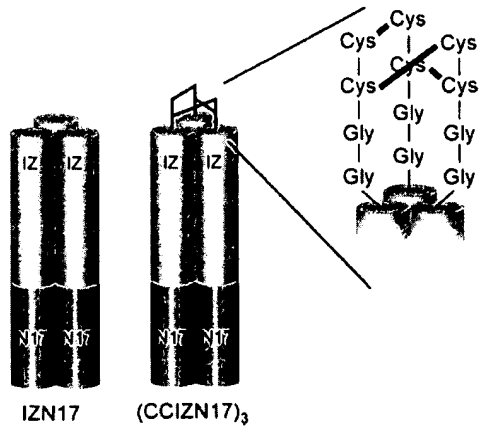

Fig. 2. Schematic model of the designed IZN17 and $(CCIZN17)_3$ molecules. The IZN17 helices forming a homotrimeric coiled-coil are represented by cylinders. Each chimeric N peptide consists of an N-terminal designed trimeric coiled-coil (light gray) fused to a portion of the sequence from the N-helix region of gp41 (dark gray), namely N17 (residues 568–584 of HIV_{HXB2}). The $(CCIZN17)_3$ helices are covalently stabilized at the N terminus by three interchain disulfides between each pair of cysteines of each peptide chain. Only one of the possible combinations of the three disulfides is drawn.

The covalent trimer $(CCIZN17)_3$ is fully helical, with a CD spectrum superimposable to that of IZN17 (Fig. 6). Thermal denaturation experiments show that, in the presence of 2 M guanidine hydrochloride, $(CCIZN17)_3$ has a T_m that is >90°C, compared with a T_m of 61.5°C for IZN17 (Table 2 and Fig. 1). It appears, therefore, that covalent stabilization of the IZN17 trimer yields a molecule with an extraordinary thermodynamic stability.

$(CCIZN17)_3$ Is an Extremely Potent Inhibitor of Viral Infectivity. We next tested $(CCIZN17)_3$ in a single-cycle infectivity assay using

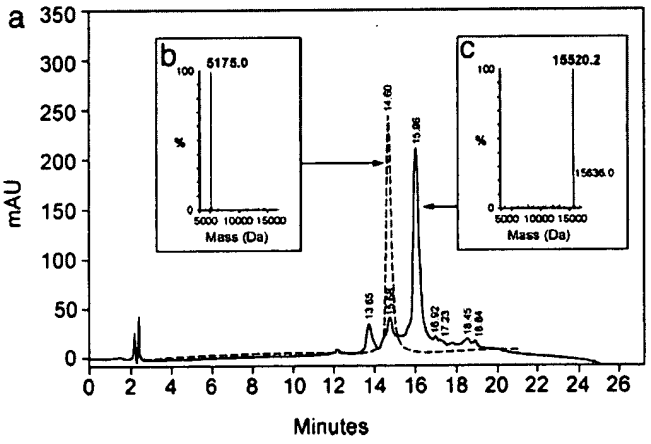

Fig. 3. Formation of the covalent trimer $(CCIZN17)_3$ monitored by HPLC-MS. (*a*) Overlay of the chromatograms of the precursor CCIZN17 (dashed line) and of the oxidized $(CCIZN17)_3$ after 18 h of incubation (solid line). (*b* and *c*) Hypermass reconstruction of the raw electrospray MS data of monomeric CCIZN17 (observed mass, 5,175.0 Da; calculated mass, 5,175.0 Da) (*b*) and the covalent trimer $(CCIZN17)_3$, whose observed mass of 15,520.1 Da (calculated mass, 15,520.2 Da), is in agreement with the formation of three disulfide bonds (*c*).

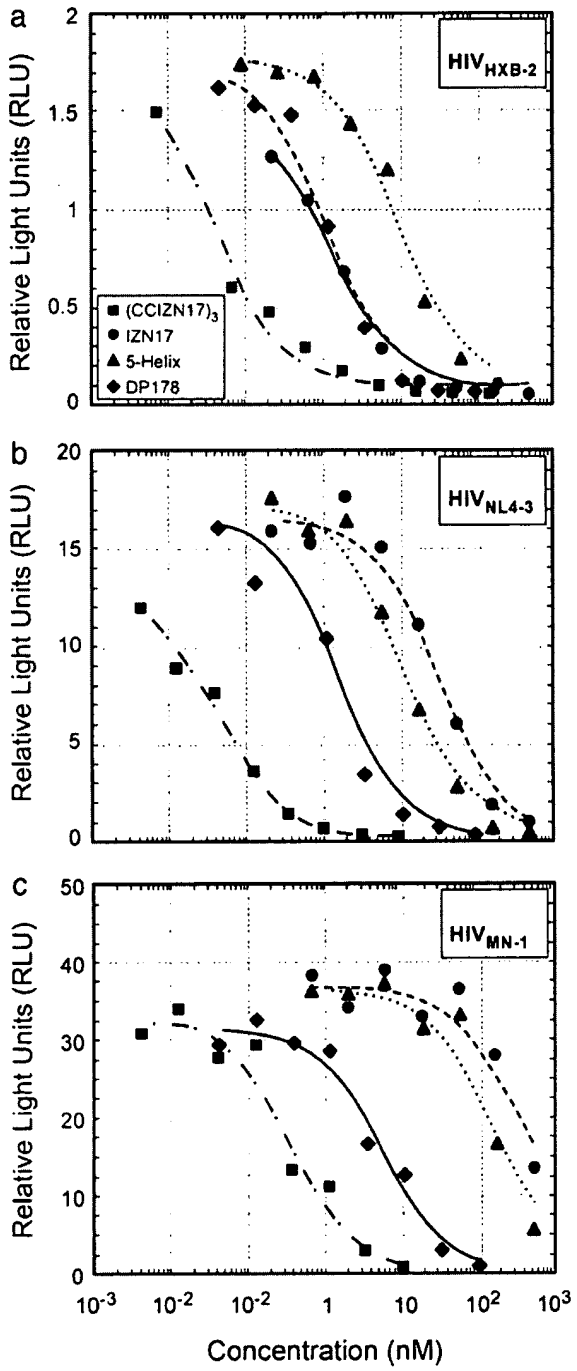

Fig. 4. Inhibition of infectivity of various HIV isolates. The calculated IC_{50} values for (CCIZN17)$_3$, IZN17, 5-helix, and DP178 are (respectively) 0.04, 1.3, 9.9, and 1.2 nM (against HIV$_{HXB2}$) (*a*); 0.046, 33.66, 12.0, and 1.7 nM (against HIV$_{NL4-3}$) (*b*); and 0.38, 394.7, 159.4, and 5.5 nM (against HIV$_{MN-1}$) (*c*).

three viral isolates (HIV$_{HXB2}$, HIV$_{NL4-3}$, and HIV$_{MN-1}$) and compared its activity with other N-peptide inhibitors and DP178, which is a C-peptide inhibitor (Table 2 and Fig. 4). (CCIZN17)$_3$ displayed subnanomolar activity against all three strains (IC_{50} values of 0.04, 0.05, and 0.38 nM, respectively) thus being 30- to 700-fold more potent than the parent peptide IZN17 (IC_{50} values of 1.3, 33.7, and 394.7 nM, respectively). (CCIZN17)$_3$ was also 250- to 400-fold more potent than 5-helix (IC_{50} values of 9.9, 12.0, and 159.4 nM, respectively). Its antiviral activity was also greater than the potent C-peptide drug DP178 (IC_{50} values of 1.2, 1.7, and 5.5 nM, respectively). Notably, (CCIZN17)$_3$ potently blocks infection by the primary isolate HIV$_{89.6}$ (IC_{50} = 5.13 nM), which is completely resistant to IZN17 and only poorly neutralized by 5-helix (IC_{50} = 213 nM).

(CCIZN17)$_3$ was then tested in a PBMC infectivity assay. The results in Table 3 are shown as IC_{95} (nM) for the three primary isolates HIV$_{89.6}$, HIV$_{BaL}$, and HIV$_{JR-FL}$ and the lab-adapted virus, HIV$_{H9IIIb}$. The assay was performed in PBMCs from two different healthy donors, and results were in good agreement. (CCIZN17)$_3$ potently neutralized three of four viruses, with an IC_{95} of one order of magnitude lower than that of IZN17, and it was also somewhat more potent than DP178. The primary isolate HIV$_{89.6}$ was not inhibited by IZN17, barely inhibited by (CCIZN17)$_3$, and only weakly inhibited by DP178 (Table 3).

(CCIZN17)$_3$ Is a Picomolar Inhibitor of HIV Fusion. We next examined the ability of (CCIZN17)$_3$ to interfere with the fusion process in a cell–cell fusion assay and compared its activity to DP178. (CCIZN17)$_3$ potently inhibits entry at subnanomolar concentrations (IC_{50} = 260 pM), showing a 55-fold higher potency than DP178 (IC_{50} = 14.5 nM) (Fig. 5).

(CCIZN17)$_3$ Displays a Broad Antiviral Profile. To determine the breadth of neutralization of (CCIZN17)$_3$, the peptide was tested in a commercial phenotypic virus assay (ViroLogic) (23) against a large panel of HIV isolates (Table 4, which is published as supporting information on the PNAS web site). The panel included R5, X4, and R5/X4 isolates. Remarkably, (CCIZN17)$_3$ was able to neutralize 100% of the strains tested, with potencies between 3.9 and 202 nM. This variable susceptibility to inhibition is comparable with what had been observed for the DP178: among 14 viral isolates tested, susceptibility varied over an ≈30-fold range (24). It is now being recognized that resistance to fusion inhibitors is complex (27, 28) and can derive from multiple factors other than the sequence of the gp41 HR (29, 30). Accordingly, the strains in Table 4, which are more resistant to (CCIZN17)$_3$, are also more resistant to known neutralizing antibodies to gp120 and gp41 (M.D.M. and R.G., unpublished data).

Combination of (CCIZN17)$_3$ with the C-Peptide Inhibitor DP178. Because (CCIZN17)$_3$ and DP178 target different regions of the prehairpin intermediate and because DP178 does not include the HR2 region, which binds to the hydrophobic pocket of N17 (6), the two inhibitors should work independently and could in principle be administered in combination. Combination therapy has been shown to be particularly effective in minimizing the emergence of resistant viruses, which are quickly selected during the course of therapy with a single drug (31). Therefore, we performed an experiment in which the two fusion inhibitors were administrated separately and in combination to assess their individual and joint effects in a spread assay in MT4 cells. The joint effect is defined as antagonistic, additive, or synergistic when the response to the combination is respectively worse, the same, or better than the expected additive response, respectively.

For (CCIZN17)$_3$ and DP178, the combination showed a strong synergistic effect at most of the dose combinations and an additive effect at the marginal dose combinations, i.e., when the concentration of one inhibitor was too low for a detectable inhibition or high enough to provide almost full inhibition by itself (Table 5, which is published as supporting information on the PNAS web site).

Other Cysteine-Capped IZN Inhibitors. The strategy of covalent stabilization by interchain disulfide bridge formation was then applied to other chimerae. We first prepared the covalent trimer of IZN23, (CCIZN23)$_3$ (Table 1), which was readily formed

Table 3. Comparative inhibition of infection of PBMCs from two different donors by $(CCIZN17)_3$, IZN17, and DP178

	IC_{95}, nM							
	HIV-$1_{89.6}$		HIV-1_{BaL}		HIV-1_{H9IIIb}		HIV-1_{JR-FL}	
Inhibitor	D1	D2	D1	D2	D1	D2	D1	D2
$(CCIZN17)_3$	>500	500	63	63	15	8	63	63
IZN17	>500	>1000	>500	500	250	125	500	250
DP178	500	250	>500	250	63	15	250	125

The IC_{95} is the concentration of test compound that inhibited virus growth by at least 95% compared with untreated infected control cultures. D1, assay run in PBMC from donor 1; D2, assay run in PBMC from donor 2.

upon incubation at neutral pH of the precursor CCIZN23 (see *Supporting Materials and Methods*). This molecule is also very stable, with $T_m > 90°C$ in 2 M guanidinium hydrochloride. In the single-cycle infectivity assay, IZN23 showed an IC_{50} of 2.3 nM against HIV_{HXB2}, whereas $(CCIZN23)_3$ showed an IC_{50} of 0.31 nM (Table 2). Thus, also in this case the covalently stabilized trimeric $(CCIZN23)_3$ is more potent than the parent noncovalent inhibitor, IZN23. However the gain in potency is only 7-fold, confirming that covalent stabilization is optimally applied to the N17 portion of the N helix, which recapitulates most of the binding energy required for fusion inhibition.

IZ Is Indispensable for the Antiviral Activity of Covalently Chimeric N Peptides. We finally investigated whether covalent stabilization might compensate for reduction in the favorable packing interactions provided by a shortened IZ coiled-coil. Thus, we designed $(CCI10N17)_3$, a chimeric construct comprising the N17 residues and only 10 of the original 24 IZ residues (I10) (Table 1). Upon incubation at neutral pH, the precursor CCI10N17 oxidized to form the covalent trimer $(CCI10N17)_3$ but with a yield of only 40%, the remainder being dimeric and tetrameric species. When the precursor CCI10N17 was analyzed by CD, it was found to be only 61% helical at 15 μM concentration, unlike IZN17 which is fully helical under the same conditions (Fig. 7, which is published as supporting information on the PNAS web site). This result confirms that the scaffold length is critical for the formation of non-covalently-stabilized, chimeric N-peptide coiled-coils. Moreover, the covalent trimer $(CCI10N17)_3$ is only 85% helical (Fig. 7), with a thermal denaturation curve showing poor cooperativity, and a T_m of only 60°C (Table 2). Overall, the data indicate that, in this case, covalent trimerization via a cysteine cap provides only marginal stabilization to the helical structure (from 61% to 85%) and, thus, that a longer scaffold is essential to nucleate a stable trimer. Consistent with previous observations, $(CCI10N17)_3$ is a much less potent HIV inhibitor than $(CCIZN17)_3$, with an IC_{50} of only 19 nM against HIV_{HXB2} (Table 2).

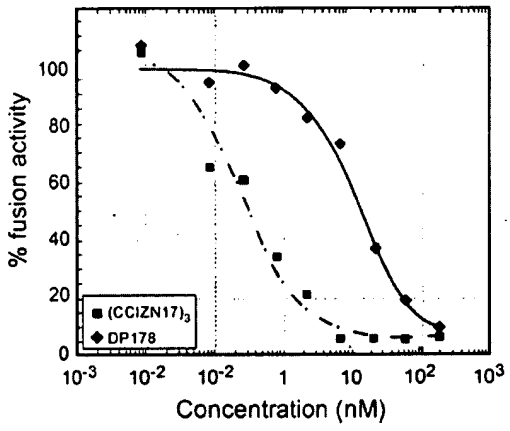

Fig. 5. Inhibition of HIV fusion in a cell–cell fusion assay. The calculated IC_{50} values for $(CCIZN17)_3$ and DP178 are 0.26 nM and 14.5 nM, respectively.

Discussion

When suitably stabilized in a coiled-coil conformation, N peptides are potent inhibitors of HIV-1 entry, with IC_{50} values in the low nanomolar range (11, 15–17). In this study, we focused our attention on the potent chimeric IZN17 peptide described by Eckert and Kim (11). The N17 sequence is the portion of the N region that comprises the gp41 hydrophobic pocket (6, 23), the region within HR1 that provides the most important set of contacts for binding of the cognate HR2 domain. Accordingly, within the IZN peptide series, IZN17 recapitulates all of the requirements for inhibitory potency, because addition of more gp41 residues to produce IZN23 and IZN36 does not result in better inhibitors (11).

To design a more potent inhibitor, we focused on N17 and found that the location of the scaffolding IZ domain relative to N17 (N- or C-terminal) has a dramatic impact on antiviral activity, as shown by the 800-fold loss in potency of N17IZ with respect to IZN17, despite the comparable structural stability (Table 2). We then addressed the main potential limitation of this inhibitor, i.e., the self-association equilibrium. The antiviral activity of the molecule depends on the presence of a trimeric coiled-coil structure, which is necessarily concentration-dependent. We thus designed a construct, CCIZN17, which can form a coiled-coil structure covalently stabilized by interchain disulfide bonds. Previously, disulfide-stabilized coiled-coil trimers of gp41 (16, 17) showed increased helical content with respect to N peptides that translated to nanomolar antiviral activity. Our covalent scaffolded trimeric $(CCIZN17)_3$ construct forms a highly stable fully helical structure that very efficiently inhibits cell–cell fusion. In a single-cycle infectivity assay against various HIV isolates, $(CCIZN17)_3$ shows much increased potency with respect to IZN17, with subnanomolar antiviral activity. Moreover, its activity is one order of magnitude higher than IZN17 in a PBMC infectivity assay. These data suggest that the maximum inhibitory potency displayed by the latter was indeed limited by its trimerization equilibrium in solution, which is confirmed by the gain in potency of another peptide in the IZN series, IZN23, when stabilized with a cysteine cap to yield $(CCIZN23)_3$.

All of the elements of $(CCIZN17)_3$ appear to synergize for optimal activity. The IZ domain cannot be shortened, as shown by the analog $(CCI10N17)_3$. In fact, $(CCI10N17)_3$, comprising only 10 of 24 residues of IZ, is much less stable and accordingly much less potent, with an IC_{50} of only 19 nM in the single-cycle infectivity assay.

In addition to showing extremely high antiviral potency, $(CCIZN17)_3$ displays a remarkable breadth of neutralization against a large panel of HIV isolates, being able to neutralize 100% of the strains tested in a commercial phenotypic virus

assay. These properties compare favorably with the recently approved C-peptide inhibitor DP178 (also known as Enfuvirtide and Fuzeon). In a variety of assays, $(CCIZN17)_3$ was equally or more potent than DP178. More importantly, we were able to demonstrate that the two inhibitors, which have different targets in gp41, synergize when used in combination. The use of two potent fusion inhibitors in combination should minimize the risk that viruses resistant to DP178 (10) or $(CCIZN17)_3$ would emerge. Taken together, these features make $(CCIZN17)_3$ an attractive lead to develop as an antiviral drug, alone or in combination with DP178.

In addition, the cysteine-capped inhibitors and $(CCIZN17)_3$ in particular likely represent faithful mimetics of at least one of the possible conformations of gp41 coiled-coil during the HIV fusion process. As such, cysteine-capped inhibitors and $(CCIZN17)_3$ may also find use as selector molecules in screening campaigns for small-molecule fusion inhibitors (26, 32–34) and as immunogens to elicit a neutralizing antibody response targeting the HIV fusion intermediate(s) (17, 35, 36). The data of Louis *et al.* (17) argue well for the latter strategy.

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Covalent stabilization of coiled coils of the HIV gp41 N region yields extremely potent and broad inhibitors of viral infection

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FUZEON™

(enfuvirtide)

for Injection

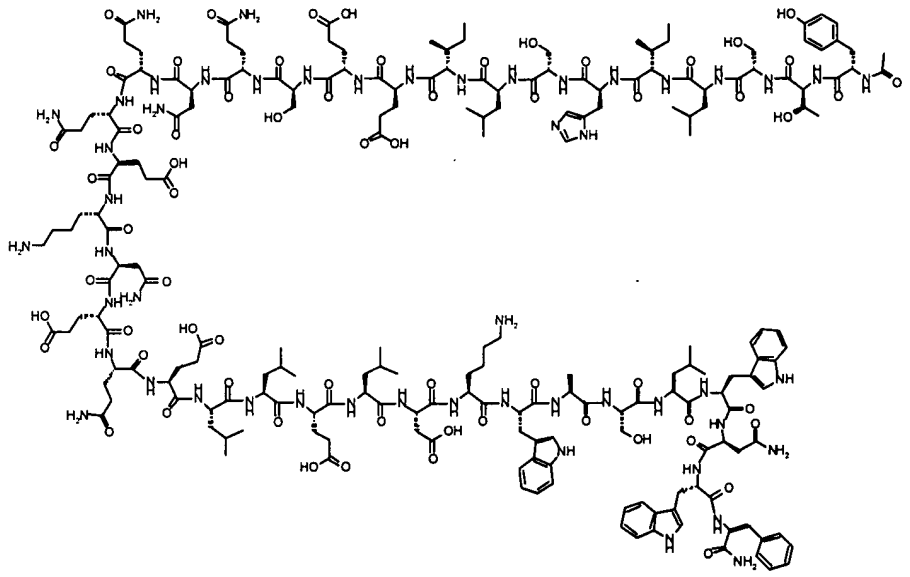
DESCRIPTION

FUZEON (enfuvirtide) is an inhibitor of the fusion of HIV-1 with CD4+ cells. Enfuvirtide is a linear 36-amino acid synthetic peptide with the N-terminus acetylated and the C-terminus is a carboxamide. It is composed of naturally occurring L-amino acid residues.

Enfuvirtide is a white to off-white amorphous solid. It has negligible solubility in pure water and the solubility increases in aqueous buffers (pH 7.5) to 85-142 g/100 mL. The empirical formula of enfuvirtide is $C_{204}H_{301}N_{51}O_{64}$, and the molecular weight is 4492. It has the following primary amino acid sequence:

CH_3CO-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH_2

and the following structural formula:



The drug product, FUZEON (enfuvirtide) for Injection, is a white to off-white, sterile, lyophilized powder. Each single-use vial contains 108 mg of enfuvirtide for the delivery of 90 mg. Prior to subcutaneous administration, the contents of the vial are reconstituted with 1.1 mL of Sterile Water for Injection giving a volume of approximately 1.2 mL to provide the delivery of 1 mL of the solution. Each 1 mL of the reconstituted solution

contains approximately 90 mg of enfuvirtide with approximate amounts of the following excipients: 22.55 mg of mannitol, 2.39 mg of sodium carbonate (anhydrous), and sodium hydroxide and hydrochloric acid for pH adjustment as needed. The reconstituted solution has an approximate pH of 9.0.

MICROBIOLOGY

Mechanism of Action

Enfuvirtide interferes with the entry of HIV-1 into cells by inhibiting fusion of viral and cellular membranes. Enfuvirtide binds to the first heptad-repeat (HR1) in the gp41subunit of the viral envelope glycoprotein and prevents the conformational changes required for the fusion of viral and cellular membranes.

Antiviral Activity In Vitro

The in vitro antiviral activity of enfuvirtide was assessed by infecting different CD4+ cell types with laboratory and clinical isolates of HIV-1. The IC_{50} (50% inhibitory concentration) for enfuvirtide in laboratory and primary isolates representing HIV-1 clades A to G ranged from 4 to 280 nM (18 to 1260 ng/mL). The IC_{50} for baseline clinical isolates ranged from 0.089 to 107 nM (0.4 to 480 ng/mL) by the cMAGI assay (n=130) and from 1.56 to 1680 nM (7 to 7530 ng/mL) by a recombinant phenotypic entry assay (n=612). Enfuvirtide was similarly active in vitro against R5, X4, and dual tropic viruses. Enfuvirtide has no activity against HIV-2.

Enfuvirtide exhibited additive to synergistic effects in cell culture assays when combined with individual members of various antiretroviral classes, including zidovudine, lamivudine, nelfinavir, indinavir, and efavirenz.

Drug Resistance

HIV-1 isolates with reduced susceptibility to enfuvirtide have been selected in vitro. Genotypic analysis of the in vitro-selected resistant isolates showed mutations that resulted in amino acid substitutions at the enfuvirtide binding HR1 domain positions 36 to 38 of the HIV-1 envelope glycoprotein gp41. Phenotypic analysis of site-directed mutants in positions 36 to 38 in an HIV-1 molecular clone showed a 5-fold to 684-fold decrease in susceptibility to enfuvirtide.

In clinical trials, HIV-1 isolates with reduced susceptibility to enfuvirtide have been recovered from subjects treated with FUZEON in combination with other antiretroviral agents. Posttreatment HIV-1 virus from 185 subjects exhibited decreases in susceptibility to enfuvirtide ranging from 4-fold to 422-fold relative to their respective baseline virus and exhibited genotypic changes in gp41 amino acids 36 to 45. Substitutions in this region were observed with decreasing frequency at amino acid positions 38, 43, 36, 40, 42, and 45.

Cross-resistance

HIV-1 clinical isolates resistant to nucleoside analogue reverse transcriptase inhibitors (NRTI), non-nucleoside analogue reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI) were susceptible to enfuvirtide in cell culture.

CLINICAL PHARMACOLOGY

Pharmacokinetics

The pharmacokinetic properties of enfuvirtide were evaluated in HIV-1 infected adult and pediatric patients.

Absorption

Following a 90-mg single subcutaneous injection of FUZEON into the abdomen in 12 HIV-1 infected subjects, the mean (±SD) C_{max} was 4.59 ± 1.5 μg/mL, AUC was 55.8 ± 12.1 μg•h/mL and the median T_{max} was 8 hours (ranged from 3 to 12 h). The absolute bioavailability (using a 90-mg intravenous dose as a reference) was 84.3% ± 15.5%. Following 90-mg bid dosing of FUZEON subcutaneously in combination with other antiretroviral agents in 11 HIV-1 infected subjects, the mean (±SD) steady-state C_{max} was 5.0 ± 1.7 μg/mL, C_{trough} was 3.3 ± 1.6 μg/mL, $AUC_{0\text{-}12h}$ was 48.7 ± 19.1 μg•h/mL, and the median T_{max} was 4 hours (ranged from 4 to 8 h).

Absorption of the 90-mg dose was comparable when injected into the subcutaneous tissue of the abdomen, thigh or arm.

Distribution

The mean (±SD) steady-state volume of distribution after intravenous administration of a 90-mg dose of FUZEON (N=12) was 5.5 ± 1.1 L.

Enfuvirtide is approximately 92% bound to plasma proteins in HIV-infected plasma over a concentration range of 2 to 10 μg/mL. It is bound predominantly to albumin and to a lower extent to α-1 acid glycoprotein.

Metabolism/Elimination

As a peptide, enfuvirtide is expected to undergo catabolism to its constituent amino acids, with subsequent recycling of the amino acids in the body pool.

Mass balance studies to determine elimination pathway(s) of enfuvirtide have not been performed in humans.

In vitro studies with human microsomes and hepatocytes indicate that enfuvirtide undergoes hydrolysis to form a deamidated metabolite at the C-terminal phenylalanine residue, M3. The hydrolysis reaction is not NADPH dependent. The M3 metabolite is detected in human plasma following administration of enfuvirtide, with an AUC ranging from 2.4% to 15% of the enfuvirtide AUC.

Following a 90-mg single subcutaneous dose of enfuvirtide (N=12) the mean ±SD elimination half-life of enfuvirtide is 3.8 ± 0.6 h and the mean ±SD apparent clearance

was 24.8 ± 4.1 mL/h/kg. Following 90-mg bid dosing of FUZEON subcutaneously in combination with other antiretroviral agents in 11 HIV-1 infected subjects, the mean ±SD apparent clearance was 30.6 ± 10.6 mL/h/kg.

Special Populations

Hepatic Insufficiency

Formal pharmacokinetic studies of enfuvirtide have not been conducted in patients with hepatic impairment.

Renal Insufficiency

Formal pharmacokinetic studies of enfuvirtide have not been conducted in patients with renal insufficiency. However, analysis of plasma concentration data from subjects in clinical trials indicated that the clearance of enfuvirtide is not affected in patients with creatinine clearance greater than 35 mL/min. The effect of creatinine clearance less than 35 mL/min on enfuvirtide clearance is unknown.

Gender and Weight

GENDER

Analysis of plasma concentration data from subjects in clinical trials indicated that the clearance of enfuvirtide is 20% lower in females than males after adjusting for body weight.

WEIGHT

Enfuvirtide clearance decreases with decreased body weight irrespective of gender. Relative to the clearance of a 70-kg male, a 40-kg male will have 20% lower clearance and a 110-kg male will have a 26% higher clearance. Relative to a 70-kg male, a 40-kg female will have a 36% lower clearance and a 110-kg female will have the same clearance.

No dose adjustment is recommended for weight or gender.

Race

Analysis of plasma concentration data from subjects in clinical trials indicated that the clearance of enfuvirtide was not different in Blacks compared to Caucasians. Other pharmacokinetic studies suggest no difference between Asians and Caucasians after adjusting for body weight.

Pediatric Patients

The pharmacokinetics of enfuvirtide have been studied in 18 pediatric subjects aged 6 through 16 years at a dose of 2 mg/kg. Enfuvirtide pharmacokinetics were determined in the presence of concomitant medications including antiretroviral agents. A dose of 2 mg/kg bid (maximum 90 mg bid) provided enfuvirtide plasma concentrations similar to those obtained in adult patients receiving 90 mg bid.

In the 18 pediatric subjects receiving the 2 mg/kg bid dose, the mean ±SD steady-state AUC was 53.6 ± 21.4 μg•h/mL, C_{max} was 5.9 ± 2.2 μg/mL, C_{trough} was 3.0 ± 1.5 μg/mL, and apparent clearance was 40 ± 14 mL/h/kg.

Geriatric Patients

The pharmacokinetics of enfuvirtide have not been studied in patients over 65 years of age.

Drug Interactions

Influence of FUZEON on the Metabolism of Concomitant Drugs

Based on the results from an in vitro human microsomal study, enfuvirtide is not an inhibitor of CYP450 enzymes. In an in vivo human metabolism study (N=12), FUZEON at the recommended dose of 90 mg bid did not alter the metabolism of CYP3A4, CYP2D6, CYP1A2, CYP2C19 or CYP2E1 substrates.

Influence of Concomitant Drugs on the Metabolism of Enfuvirtide

In separate pharmacokinetic interaction studies, coadministration of ritonavir (N=12), saquinavir/ritonavir (N=12), and rifampin (N=12) did not result in clinically significant pharmacokinetic interactions with FUZEON (see Table 1).

Table 1. Effect of Ritonavir, Saquinavir/Ritonavir, and Rifampin on the Steady-State Pharmacokinetics of Enfuvirtide (90 mg bid)*

Coadministered Drug	Dose of Coadministered Drug	N	% Change of Enfuvirtide Pharmacokinetic Parameters† (90% CI)		
			C_{max}	AUC	C_{trough}
Ritonavir	200 mg, q12h, 4 days	12	↑24 (↑9 to ↑41)	↑22 (↑8 to ↑37)	↑14 (↑2 to ↑28)
Saquinavir/ Ritonavir	1000/100 mg, q12h, 4 days	12	⇔	↑14 (↑5 to ↑24)	↑26 (↑17 to↑35)
Rifampin	600 mg, qd, 10 days	12	⇔	⇔	↓15 (↓22 to ↓7)

* All studies were performed in HIV-1+ subjects using a sequential crossover design.
† ↑= Increase; ↓ = Decrease; ⇔ = No Effect (↑ or ↓ <10%)

INDICATIONS AND USAGE

FUZEON in combination with other antiretroviral agents is indicated for the treatment of HIV-1 infection in treatment-experienced patients with evidence of HIV-1 replication despite ongoing antiretroviral therapy.

This indication is based on analyses of plasma HIV-1 RNA levels and CD4 cell counts in controlled studies of FUZEON of 24 weeks duration. Subjects enrolled were treatment-experienced adults; many had advanced disease. There are no studies of FUZEON in

antiretroviral naive patients. There are no results from controlled trials evaluating the effect of FUZEON on clinical progression of HIV-1.

Description of Clinical Studies

Studies in Antiretroviral Experienced Patients

Studies T20-301 and T20-302 are ongoing, randomized, controlled, open-label, multicenter trials in HIV-1 infected subjects. Subjects were required to have either (1) viremia despite 3 to 6 months prior therapy with a nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), and protease inhibitor (PI) or (2) viremia and documented resistance or intolerance to at least one member in each of the NRTI, NNRTI, and PI classes.

All subjects received an individualized background regimen consisting of 3 to 5 antiretroviral agents selected on the basis of the subject's prior treatment history and baseline genotypic and phenotypic viral resistance measurements. Subjects were then randomized at a 2:1 ratio to FUZEON 90 mg bid with background regimen or background regimen alone.

Demographic characteristics for studies T20-301 and T20-302 are shown in Table 2. Subjects had prior exposure to a median of 12 antiretrovirals for a median of 7 years.

Table 2. T20-301 and T20-302 Pooled Subject Demographics

	FUZEON+Background Regimen	**Background Regimen**
	N=661	**N=334**
Sex		
Male	90%	90%
Female	10%	10%
Race		
White	89%	89%
Black	8%	7%
Mean Age (yr) (range)	43 (16-67)	43 (24-82)
Median Baseline HIV-1 RNA (log_{10} copies/mL)	5.2 (3.5-6.7)	5.1 (3.7-7.1)
Median Baseline CD4 Cell Count (cells/mm^3)	88 (1-994)	97 (1-847)

The change in plasma HIV-1 RNA from baseline to week 24 was –1.52 log_{10} copies/mL for subjects receiving FUZEON plus background regimen compared to –0.73 log_{10} copies/mL for subjects receiving the background regimen only (see Table 3).

Subjects with two or more active drugs in their background regimen were more likely to achieve a HIV-1 RNA of <400 copies/mL.

Table 3. Outcomes of Randomized Treatment at Week 24 (Pooled Studies T20-301 and T20-302)

Outcomes	FUZEON +Background Regimen 90 mg bid	Background Regimen
	N=661	N=334
HIV-1 RNA Log Change from Baseline (log_{10} copies/mL)*	-1.52	-0.73
CD4+ cell count Change from Baseline (cells/mm^3)#	+71	+35
HIV RNA ≥1 log below Baseline	342 (52%)	86 (26%)
HIV RNA <400 copies/mL	247 (37%)	54 (16%)
HIV RNA <50 copies/mL	151 (23%)	30 (9%)
Discontinued due to adverse reactions/labs†	40 (6%)	12 (4%)
Discontinued due to injection site reactions†	20 (3%)	N/A
Discontinued due to other reasons†ϕ§	36 (5%)	14 (4%)

* Based on results from pooled data of T20-301 and T20-302 on ITT population (week 24 viral load for subjects who were lost to follow-up, discontinued therapy, or switched from their original randomization, is replaced by their baseline value).

Last value carried forward

† Percentages based on safety population FUZEON+background (N=663) and background (N=337).

ϕ As per the judgment of the investigator.

§ Includes discontinuations from loss to follow-up, treatment refusal, and other reasons.

CONTRAINDICATIONS

FUZEON is contraindicated in patients with known hypersensitivity to FUZEON or any of its components (see WARNINGS).

WARNINGS

Local Injection Site Reactions

The most common adverse events associated with FUZEON use are local injection site reactions. Manifestations may include pain and discomfort, induration, erythema, nodules and cysts, pruritus, and ecchymosis. Nine percent of patients had local reactions that required analgesics or limited usual activities (see ADVERSE REACTIONS). Reactions are often present at more than one injection site. Patients must be familiar with the FUZEON *Injection Instructions* in order to know how to inject FUZEON appropriately and how to monitor carefully for signs or symptoms of cellulitis or local infection.

Pneumonia

An increased rate of bacterial pneumonia was observed in subjects treated with FUZEON in the Phase 3 clinical trials compared to the control arm (see ADVERSE REACTIONS). It is unclear if the increased incidence of pneumonia is related to FUZEON use. However, because of this finding, patients with HIV infection should be carefully monitored for signs and symptoms of pneumonia, especially if they have underlying conditions which may predispose them to pneumonia. Risk factors for pneumonia included low initial CD4 cell count, high initial viral load, intravenous drug use, smoking, and a prior history of lung disease (see ADVERSE REACTIONS).

Hypersensitivity Reactions

Hypersensitivity reactions have been associated with FUZEON therapy and may recur on re-challenge. Hypersensitivity reactions have included individually and in combination: rash, fever, nausea and vomiting, chills, rigors, hypotension, and elevated serum liver transaminases. Other adverse events that may be immune mediated and have been reported in subjects receiving FUZEON include primary immune complex reaction, respiratory distress glomerulonephritis, and Guillain-Barre syndrome. Patients developing signs and symptoms suggestive of a systemic hypersensitivity reaction should discontinue FUZEON and should seek medical evaluation immediately. Therapy with FUZEON should not be restarted following systemic signs and symptoms consistent with a hypersensitivity reaction. Risk factors that may predict the occurrence or severity of hypersensitivity to FUZEON have not been identified (see ADVERSE REACTIONS).

PRECAUTIONS

Non-HIV Infected Individuals

There is a theoretical risk that FUZEON use may lead to the production of anti-enfuvirtide antibodies which cross react with HIV gp41. This could result in a false positive HIV test with an ELISA assay; a confirmatory western blot test would be expected to be negative. FUZEON has not been studied in non-HIV infected individuals.

Information for Patients

To assure safe and effective use of FUZEON, the following information and instructions should be given to patients:

- Patients should be informed that injection site reactions occur commonly. Patients must be familiar with the FUZEON *Injection Instructions* for instructions on how to appropriately inject FUZEON and how to carefully monitor for signs or symptoms of cellulitis or local infection. Patients should be instructed when to contact their healthcare provider about these reactions.

- Patients should be made aware that an increased rate of bacterial pneumonia was observed in subjects treated with FUZEON in Phase 3 clinical trials compared to the control arm. Patients should be advised to seek medical evaluation immediately if they develop signs or symptoms suggestive of pneumonia (cough with fever, rapid breathing, shortness of breath) (see WARNINGS).

- Patients should be advised of the possibility of a hypersensitivity reaction to FUZEON. Patients should be advised to discontinue therapy and immediately seek medical evaluation if they develop signs/symptoms of hypersensitivity (see WARNINGS).
- FUZEON is not a cure for HIV-1 infection and patients may continue to contract illnesses associated with HIV-1 infection. The long-term effects of FUZEON are unknown at this time. FUZEON therapy has not been shown to reduce the risk of transmitting HIV-1 to others through sexual contact or blood contamination.
- FUZEON must be taken as part of a combination antiretroviral regimen. Use of FUZEON alone may lead to rapid development of virus resistant to FUZEON and possibly other agents of the same class.
- Patients and caregivers must be instructed in the use of aseptic technique when administering FUZEON in order to avoid injection site infections. Appropriate training for FUZEON reconstitution and self-injection must be given by a healthcare provider, including a careful review of the FUZEON Patient Package Insert and FUZEON *Injection Instructions*. The first injection should be performed under the supervision of an appropriately qualified healthcare provider. It is recommended that the patient and/or caregiver's understanding and use of aseptic self-injection techniques and procedures be periodically re-evaluated.
- Patients should contact their healthcare provider for any questions regarding the administration of FUZEON. Patients should be told not to reuse needles or syringes, and be instructed in safe disposal procedures including the use of a puncture-resistant container for disposal of used needles and syringes. Patients must be instructed on the safe disposal of full containers as per local requirements. Caregivers who experience an accidental needlestick after patient injection should contact a healthcare provider immediately.
- Patients should inform their healthcare provider if they are pregnant, plan to become pregnant or become pregnant while taking this medication.
- Patients should inform their healthcare provider if they are breast-feeding.
- Patients should not change the dose or dosing schedule of FUZEON or any antiretroviral medication without consulting their healthcare provider.
- Patients should contact their healthcare provider immediately if they stop taking FUZEON or any other drug in their antiretroviral regimen.
- Patients should be told that they can obtain more information on the self-administration of FUZEON at www.FUZEON.com or by calling 1-877-4-FUZEON (1-877-438-9366).

Patients should be advised that no studies have been conducted on the ability to drive or operate machinery while taking FUZEON. If patients experience dizziness while taking FUZEON, they should be advised to talk to their healthcare provider before driving or operating machinery.

Drug Interactions

CYP450 Metabolized Drugs

Results from in vitro and in vivo studies suggest that enfuvirtide is unlikely to have significant drug interactions with concomitantly administered drugs metabolized by CYP450 enzymes (see CLINICAL PHARMACOLOGY).

Antiretroviral Agents

No drug interactions with other antiretroviral medications have been identified that would warrant alteration of either the enfuvirtide dose or the dose of the other antiretroviral medication.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Long-term animal carcinogenicity studies of enfuvirtide have not been conducted.

Mutagenesis

Enfuvirtide was neither mutagenic nor clastogenic in a series of in vivo and in vitro assays including the Ames bacterial reverse mutation assay, a mammalian cell forward gene mutation assay in AS52 Chinese Hamster ovary cells or an in vivo mouse micronucleus assay.

Impairment of Fertility

Enfuvirtide produced no adverse effects on fertility in male or female rats at doses of up to 30 mg/kg/day administered by subcutaneous injection (1.6 times the maximum recommended adult human daily dose on a m^2 basis).

Pregnancy

Pregnancy Category B. Reproduction studies have been performed in rats and rabbits at doses up to 27 times and 3.2 times the adult human dose on a m^2 basis. The animal studies revealed no evidence of harm to the fetus from enfuvirtide. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Antiretroviral Pregnancy Registry

To monitor maternal-fetal outcomes of pregnant women exposed to FUZEON and other antiretroviral drugs, an Antiretroviral Pregnancy Registry has been established. Physicians are encouraged to register patients by calling 1-800-258-4263.

Nursing Mothers

The Centers for Disease Control and Prevention recommends that HIV-infected mothers not breast-feed their infants to avoid the risk of postnatal transmission of HIV. It is not known whether enfuvirtide is excreted in human milk. Because of both the

potential for HIV transmission and the potential for serious adverse reactions in nursing infants, **mothers should be instructed not to breast-feed if they are receiving FUZEON.**

Studies where radio-labeled ^{3}H-enfuvirtide was administered to lactating rats indicated that radioactivity was present in the milk. It is not known whether the radioactivity in the milk was from radio-labeled enfuvirtide or from radio-labeled metabolites of enfuvirtide (ie, amino acids and peptide fragments).

Pediatric Use

The safety and pharmacokinetics of FUZEON have not been established in pediatric subjects below 6 years of age. Limited efficacy data is available in pediatric subjects 6 years of age and older.

Thirty-five HIV-1 infected pediatric subjects ages 6 through 16 years have received FUZEON in two open-label, single-arm clinical trials. Adverse experiences were similar to those observed in adult patients.

Study T20-204 was an open-label, multicenter trial that evaluated the safety, and antiviral activity of FUZEON in treatment-experienced pediatric subjects. Eleven subjects from 6 to 12 years were enrolled (median age of 9 years). Median baseline CD4 cell count was 509 cells/μL and the median baseline HIV-1 RNA was 4.5 $\log_{10}$ copies/mL.

Ten of the 11 study subjects completed 48 weeks of chronic therapy. By week 48, 6/11 (55%) subjects had ≥1 $\log_{10}$ decline in HIV-1 RNA and 4/11 (36%) subjects were below 400 copies/mL of HIV-1 RNA. The median changes from baseline in HIV-1 RNA and CD4 cell count were -1.48 $\log_{10}$ copies/mL and 122 cells/μL, respectively.

Study T20-310 is an ongoing, open-label, multicenter trial evaluating the pharmacokinetics, safety, and antiviral activity of FUZEON in treatment-experienced pediatric subjects and adolescents. Twenty-four subjects from 6 through 16 years were enrolled (median age of 13 years). Median baseline CD4 cell count was 143 cells/μL and the median baseline HIV-1 RNA was 5.0 $\log_{10}$ copies/mL. The evaluation of the antiviral activity is ongoing.

Geriatric Use

Clinical studies of FUZEON did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects.

ADVERSE REACTIONS

The overall safety profile of FUZEON is based on 1188 subjects who received at least 1 dose of FUZEON during various clinical trials. This includes 1153 adults, 608 of whom received the recommended dose for greater than 24 weeks, and 35 pediatric subjects.

Assessment of treatment-emergent adverse events is based on the pooled data from the two Phase 3 studies T20-301 and T20-302.

Local Injection Site Reactions

Local injection site reactions were the most frequent adverse events associated with the use of FUZEON. In Phase 3 clinical studies (T20-301 and T20-302), 98% of subjects had at least 1 local injection site reaction (ISR). Three percent of subjects discontinued treatment with FUZEON because of ISRs. Eighty-six percent of subjects experienced their first ISR during the initial week of treatment. The majority of ISRs were associated with mild to moderate pain at the injection site, erythema, induration, and the presence of nodules or cysts. For most subjects the severity of signs and symptoms associated with ISRs did not change during the 24 weeks of treatment. In 17% of subjects an individual ISR lasted for longer than 7 days. Because of the frequency and duration of individual ISRs, 23% of subjects had six or more ongoing ISRs at any given time. Individual signs and symptoms characterizing local ISRs are summarized in Table 4. Infection at the injection site (including abscess and cellulitis) was reported in 1% of subjects.

Table 4. Summary of Individual Signs/Symptoms Characterizing Local Injection Site Reactions to Enfuvirtide in Studies T20-301 and T20-302 Combined (% of Subjects)

	N=663		
Event Category	**Any Severity Grade**	**% of Events Comprising Grade 3 Reactions**	**% of Events Comprising Grade 4 Reactions**
Pain/Discomfort[a]	95%	9%	0%
Induration[b]	89%	41%	16%
Erythema[c]	89%	22%	10%
Nodules and Cysts[d]	76%	26%	0%
Pruritus[e]	62%	4%	NA
Ecchymosis[f]	48%	8%	5%

[a] Grade 3 = severe pain requiring analgesics (or narcotic analgesics for ≤72 hours) and/or limiting usual activities;
Grade 4 = severe pain requiring hospitalization or prolongation of hospitalization, resulting in death, or persistent or significant disability/incapacity, or life-threatening, or medically significant.

[b] Grade 3 = ≥25 mm but <50 mm; Grade 4 = ≥50 mm average diameter.

[c] Grade 3 = ≥50 mm but <85 mm average diameter; Grade 4 = ≥85 mm average diameter.

[d] Grade 3 = ≥3 cm; Grade 4 = if draining.

[e] Grade 3 = refractory to topical treatment or requiring oral or parenteral treatment; Grade 4 = not applicable.

[f] Grade 3 = >3 cm but ≤5 cm; Grade 4 = >5 cm.

Other Adverse Events

Hypersensitivity reactions have been attributed to FUZEON (≤1%) and in some cases have recurred upon re-challenge (see WARNINGS).

The events most frequently reported in subjects receiving FUZEON+background regimen, excluding injection site reactions, were diarrhea (26.8%), nausea (20.1%), and fatigue (16.1%). These events were also commonly observed in subjects that received background regimen alone: diarrhea (33.5%), nausea (23.7%), and fatigue (17.4%).

Treatment-emergent adverse events (% of subjects), excluding ISRs, from Phase 3 studies are summarized for adult subjects, regardless of severity and causality, in Table 5. Only events occurring in ≥2% of subjects and at a higher rate in subjects treated with FUZEON are summarized in Table 5; events that occurred at a higher rate in the control arms are not displayed.

Table 5. Percentage of Patients With Selected Treatment-Emergent Adverse Events* Reported in ≥2% of Adult Patients and Occurring More Frequently in Patients Treated With FUZEON (Pooled Studies T20-301/T20-302 at 24 Weeks)

Adverse Event (by System Organ Class)	FUZEON+ Background Regimen	Background Regimen
	N=663	N=334
Nervous System Disorders		
Peripheral Neuropathy	8.9%	6.3%
Taste Disturbance	2.4%	1.5%
Psychiatric Disorders		
Insomnia	11.3%	8.7%
Depression	8.6%	7.2%
Anxiety	5.7%	3.0%
Respiratory, Thoracic, and Mediastinal Disorders		
Cough	7.4%	5.4%
Infections		
Sinusitis	6.2%	2.1%
Herpes Simplex	5.0%	3.9%
Skin Papilloma	4.2%	1.5%
Influenza	3.9%	1.8%
General		
Weight Decreased	6.5%	5.1%
Appetite Decreased	6.3%	2.4%
Asthenia	5.7%	4.2%
Anorexia	2.6%	1.8%
Influenza-like Illness	2.3%	0.9%
Skin and Subcutaneous Tissue Disorders		
Pruritus Nos	5.1%	4.2%
Musculoskeletal, Connective Tissue, and Bone Disorders		
Myalgia	5.0%	2.4%
Gastrointestinal Disorders		
Constipation	3.9%	2.7%
Abdominal Pain Upper	3.0%	2.7%
Pancreatitis	2.4%	0.9%
Eye Disorders		
Conjunctivitis	2.4%	0.9%
Blood and Lymphatic System Disorders		
Lymphadenopathy	2.3%	0.3%

*Excludes Injection Site Reactions

An increased rate of bacterial pneumonia was observed in subjects treated with FUZEON in the Phase 3 clinical trials compared to the control arm (4.68 pneumonia events per 100 patient-years versus 0.61 events per 100 patient-years, respectively). Approximately half

of the study subjects with pneumonia required hospitalization. One subject death in the FUZEON arm was attributed to pneumonia. Risk factors for pneumonia included low initial CD4 lymphocyte count, high initial viral load, intravenous drug use, smoking, and a prior history of lung disease. It is unclear if the increased incidence of pneumonia was related to FUZEON use. However, because of this finding patients with HIV infection should be carefully monitored for signs and symptoms of pneumonia, especially if they have underlying conditions which may predispose them to pneumonia (see WARNINGS).

Less Common Events

The following adverse events have been reported in 1 or more subjects; however, a causal relationship to FUZEON has not been established.

Immune System Disorders: worsening abacavir hypersensitivity reaction

Renal and Urinary Disorders: renal insufficiency (glomerulonephritis); renal failure

Blood and Lymphatic Disorders: thrombocytopenia; neutropenia, and fever

Endocrine and Metabolic: hyperglycemia

Infections and Infestations: pneumonia

Nervous System Disorders: Guillain-Barre syndrome (fatal); sixth nerve palsy

Laboratory Abnormalities

Table 6 shows the treatment-emergent laboratory abnormalities that occurred in at least 2% of subjects and more frequently in those receiving FUZEON+background regimen than background regimen alone from studies T20-301 and T20-302.

Table 6. Percentage of Treatment-Emergent Laboratory Abnormalities That Occurred in ≥2% of Adult Patients and More Frequently in Patients Receiving FUZEON (Pooled Studies T20-301 and T20-302 at 24 Weeks)

Laboratory Parameters	Grading	FUZEON+ Background Regimen	Background Regimen
		N=663	N=334
Eosinophilia			
1-2 X ULN (0.7 x 10^9/L)	0.7-1.4 x 10^9/L	8.3%	1.5%
>2 X ULN(0.7 x 10^9/L)	>1.4 x 10^9/L	1.8%	0.9%
Amylase (U/L)			
Gr. 3	>2-5 x ULN	6.2%	3.6%
Gr. 4	>5 x ULN or clinical pancreatitis	0.9%	0.6%
Lipase (U/L)			
Gr. 3	>2-5 x ULN	5.9%	3.6%
Gr. 4	>5 x ULN	2.3%	1.8%
Triglycerides (mmol/L)			
Gr. 3	>1000 mg/dL	8.9%	7.2%
ALT			
Gr. 3	>5-10 x ULN	3.5%	2.1%
Gr. 4	>10 x ULN	0.9%	0.6%
AST			
Gr. 3	>5-10 x ULN	3.6%	3.0%
Gr. 4	>10 x ULN	1.2%	0.6%
Creatine Phosphokinase (U/L)			
Gr. 3	>5-10 x ULN	5.9%	3.6%
Gr. 4	>10 x ULN	2.3%	3.6%
GGT (U/L)			
Gr. 3	>5-10 x ULN	3.5%	3.3%
Gr. 4	>10 x ULN	2.4%	1.8%
Hemoglobin (g/dL)			
Gr. 3	6.5-7.9 g/dL	1.5%	0.9%
Gr. 4	<6.5 g/dL	0.6%	0.6%

Adverse Events in Pediatric Patients

FUZEON has been studied in 35 pediatric subjects 6 through 16 years of age with duration of FUZEON exposure ranging from 1 dose to 48 weeks. Adverse experiences seen during clinical trials were similar to those observed in adult subjects.

OVERDOSAGE

There are no reports of human experience of acute overdose with FUZEON. The highest dose administered to 12 subjects in a clinical trial was 180 mg as a single dose subcutaneously. There is no specific antidote for overdose with FUZEON. Treatment of overdose should consist of general supportive measures.

DOSAGE AND ADMINISTRATION

Adults

The recommended dose of FUZEON is 90 mg (1 mL) twice daily injected subcutaneously into the upper arm, anterior thigh or abdomen. Each injection should be given at a site different from the preceding injection site, and only where there is no current injection site reaction from an earlier dose. FUZEON should not be injected into moles, scar tissue, bruises or the navel. Additional detailed information regarding the administration of FUZEON is described in the FUZEON *Injection Instructions*.

Pediatric Patients

No data are available to establish a dose recommendation of FUZEON in pediatric patients below the age of 6 years. In pediatric patients 6 years through 16 years of age, the recommended dosage of FUZEON is 2 mg/kg twice daily up to a maximum dose of 90 mg twice daily injected subcutaneously into the upper arm, anterior thigh or abdomen. Each injection should be given at a site different from the preceding injection site and only where there is no current injection site reaction from an earlier dose. FUZEON should not be injected into moles, scar tissue, bruises or the navel. Table 7 contains dosing guidelines for FUZEON based on body weight. Weight should be monitored periodically and the FUZEON dose adjusted accordingly.

Table 7. Pediatric Dosing Guidelines

Weight		Dose per bid Injection (mg/dose)	Injection Volume (90 mg enfuvirtide per mL)
Kilograms (kg)	Pounds (lbs)		
11.0 to 15.5	24 to 34	27	0.3 mL
15.6 to 20.0	>34 to 44	36	0.4 mL
20.1 to 24.5	>44 to 54	45	0.5 mL
24.6 to 29.0	>54 to 64	54	0.6 mL
29.1 to 33.5	>64 to 74	63	0.7 mL
33.6 to 38.0	>74 to 84	72	0.8 mL
38.1 to 42.5	>84 to 94	81	0.9 mL
≥42.6	>94	90	1.0 mL

Directions for Use

For more detailed instructions, see FUZEON *Injection Instructions.*

Subcutaneous Administration

FUZEON must only be reconstituted with 1.1 mL of Sterile Water for Injection. After adding sterile water, the vial should be gently tapped for 10 seconds and then gently rolled between the hands to avoid foaming and to ensure all particles of drug are in contact with the liquid and no drug remains on the vial wall. The vial should then be allowed to stand until the powder goes completely into solution, which could take up to 45 minutes. Reconstitution time can be reduced by gently rolling the vial between the hands until the product is completely dissolved. Before the solution is withdrawn for administration, the vial should be inspected visually to ensure that the contents are fully dissolved in solution, and that the solution is clear, colorless and without bubbles or particulate matter. If there is evidence of particulate matter, the vial must not be used and should be returned to the pharmacy.

FUZEON contains no preservatives. Once reconstituted, FUZEON should be injected immediately or kept refrigerated in the original vial until use. Reconstituted FUZEON must be used within 24 hours. The subsequent dose of FUZEON can be reconstituted in advance and must be stored in the refrigerator in the original vial and used within 24 hours. Refrigerated reconstituted solution should be brought to room temperature before injection and the vial should be inspected visually again to ensure that the contents are fully dissolved in solution and that the solution is clear, colorless, and without bubbles or particulate matter.

The reconstituted solution should be injected subcutaneously in the upper arm, abdomen or anterior thigh. The injection should be given at a site different from the preceding injection site and only where there is no current injection site reaction. Also, do not inject into moles, scar tissue, bruises or the navel. A vial is suitable for single use only; unused portions must be discarded (see FUZEON *Injection Instructions*).

Patients should contact their healthcare provider for any questions regarding the administration of FUZEON. Information about the self-administration of FUZEON may also be obtained by calling the toll-free number 1-877-4-FUZEON (1-877-438-9366) or at the FUZEON website, www.FUZEON.com. Patients should be taught to recognize the signs and symptoms of injection site reactions and instructed when to contact their healthcare provider about these reactions.

HOW SUPPLIED

FUZEON (enfuvirtide) for Injection is a white to off-white, sterile, lyophilized powder and it is packaged in a single-use clear glass vial containing 108 mg of enfuvirtide for the delivery of approximately 90 mg/1 mL when reconstituted with 1.1 mL of Sterile Water for Injection.

FUZEON is available in a Convenience Kit containing 60 single-use vials (2 cartons of 30 each) of FUZEON (90 mg strength), 60 vials (2 cartons of 30 each) of Sterile Water for Injection (1.1 mL per vial), 60 reconstitution syringes (3 cc), 60 administration syringes (1 cc), alcohol wipes, Package Insert, Patient Package Insert, and Injection Instruction Guide (NDC 0004-0380-39).

Storage Conditions

Store at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F) [See USP Controlled Room Temperature].

Reconstituted solution should be stored under refrigeration at 2° to 8°C (36° to 46°F) and used within 24 hours.

R_X only

FUZEON has been jointly developed by Trimeris, Inc. and Hoffmann-La Roche Inc. FUZEON is manufactured by Hoffmann-La Roche Inc.

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Information for Patients and Caregivers **(New as of March, 2003)**

FUZEON™ (few'-zee-on)

Generic Name: enfuvirtide (en-few'-ver-tide) for Injection

This leaflet contains important information for patients and their caregivers about FUZEON. Please read this leaflet and FUZEON *Injection Instructions* carefully before you start using FUZEON. Always read the section *"Changes since the last version of this leaflet"* at the end of this leaflet each time you get your FUZEON prescription refilled. There may be new important information about the use of FUZEON.

This information does not take the place of talking with your healthcare provider about your medical conditions or treatment.

What is FUZEON?

FUZEON is a medicine called an HIV (human immunodeficiency virus) fusion inhibitor. FUZEON is always used with other anti-HIV medicines to treat adults and children ages 6 years and older with HIV infection. FUZEON is not used by itself to treat HIV infection.

FUZEON blocks HIV's ability to infect healthy CD4 cells. When used with other anti-HIV medicines, FUZEON can reduce the amount of HIV in the blood and increase the number of CD4 cells. This may keep your immune system healthy, so it can help fight infection.

FUZEON does not cure HIV infection or AIDS. The long-term effects of FUZEON are not known at this time. People taking FUZEON may still get opportunistic infections or other conditions that can happen with HIV infection. **For these reasons it is very important that you remain under the care of your healthcare provider while taking FUZEON.**

Does FUZEON lower the chance of passing HIV to other people?

FUZEON does not lower your chance of passing HIV to other people through unprotected sex, sharing needles or being exposed to your blood. For your own health and the health of others, it is important to continue to practice safer sex. Use a latex or polyurethane condom or other barrier method to lower the chance of sexual contact with semen, vaginal secretions or blood. Never use or share dirty needles. Ask your healthcare provider if you have any questions about safer sex or how to prevent passing HIV to other people.

Who should not use FUZEON?

Do not use FUZEON if you are allergic to any of the ingredients in FUZEON. See the end of this leaflet for a list of ingredients in FUZEON.

Tell your healthcare provider:

- **if you are pregnant or plan to become pregnant.** We do not know if FUZEON can harm your unborn child. You and your healthcare provider will need to decide if FUZEON is right for you. If you use FUZEON while you are pregnant, talk to your healthcare provider about how you can be in the Antiretroviral Pregnancy Registry.
- **if you are breast-feeding.** You should not breast-feed if you are HIV-positive because of the chance of passing the HIV virus to your baby. Also, it is not known if FUZEON can pass into your breast milk and if it can harm your baby.
- **about all your medical conditions.**

- **about all the medicines you use,** including prescription and non-prescription medicines, vitamins, and herbal supplements. FUZEON has not been tested with all medicines.

How should I use FUZEON?

Before you use FUZEON, make sure you understand all of the information in this leaflet and the FUZEON *Injection Instructions* that come with your medicine. You or your caregiver should be trained by a healthcare provider before injecting it. If you do not understand all the information, talk with your healthcare provider about your questions or concerns.

- Use FUZEON with other anti-HIV medicines. **Do not use FUZEON as your only anti-HIV medicine.**
- FUZEON must be injected. FUZEON does not work if the medicine is swallowed.
- Do not mix other medicines in the same syringe with FUZEON.
- FUZEON is given under the skin by injection (a "shot") in the upper arm, upper leg or stomach two times a day. See the FUZEON *Injection Instructions* that come with your medicine for step-by-step instructions about how to inject FUZEON.
- Do not inject FUZEON in the same area as you did the time before. Do not inject FUZEON into the following areas: around the navel (belly button), scar tissue, a bruise or a mole, and where there is an injection site reaction.
- Do not inject FUZEON if you see particles floating in the FUZEON vial after you mix it up.
- You can use FUZEON whether you have eaten or not. Food does not affect FUZEON. However, you must keep taking your other medicines the way you did before.
- Do not change your dose or stop taking FUZEON without first talking with your healthcare provider.
- See your healthcare provider regularly while using FUZEON.
- When your FUZEON supply runs low, be sure to have it refilled. This is very important because the amount of virus in your blood may increase if the medicine is stopped for even a short time. If you miss or skip doses of FUZEON, HIV may develop resistance to FUZEON and become harder to treat.
- If you miss a dose of FUZEON, take the missed dose as soon as you can and then take your next dose as scheduled. If you have missed a dose of FUZEON and it is close to the time when you are supposed to take your next dose, wait and take the next dose as regularly scheduled. Do not take two doses of FUZEON at the same time.

- If you take too much FUZEON, call your healthcare provider right away. We do not know what can happen if you take too much FUZEON. You will be watched very carefully if you take too much FUZEON.
- **It is important that you put your used syringes into a special sharps container after injecting FUZEON.** Your healthcare provider will give you more instructions about the safe disposal of your used syringes. **Do not put them in a trash can.** If you do not have a sharps container, call your healthcare provider or pharmacist to get one before using FUZEON.

Can FUZEON be used with other medicines?

- FUZEON does not affect other anti-HIV medicines or the medicine rifampin (also known as rifampicin and manufactured under the brand names Rifadin® and Rimactane®). You can take FUZEON at the same times or at different times than your other anti-HIV medicines.

What should I avoid while using FUZEON?

- Avoid doing anything that can spread HIV infection since FUZEON does not stop you from passing the HIV infection to others.
- Do not share needles or other injection equipment.
- Do not share personal items that can have blood or body fluids on them, like toothbrushes or razor blades.
- Do not have any kind of sex without protection. Always practice safer sex by using a latex or polyurethane condom or other barrier method to reduce the chance of sexual contact with semen, vaginal secretions or blood.
- Do not drive or operate heavy machinery if FUZEON makes you feel dizzy.

What are the possible side effects of FUZEON?

Injection site reactions

FUZEON causes injection site reactions. Almost all people get injection site reactions with FUZEON. Reactions are usually mild to moderate but occasionally may be severe. Reactions on the skin where FUZEON is injected include:

- itching
- swelling
- redness
- pain or tenderness
- hardened skin
- bumps

These reactions generally happen within the first week of FUZEON treatment and usually happen again as you keep using FUZEON. A reaction at one skin injection site usually lasts for less than 7 days.

Injection site reactions may be worse when injections are given again in the same place on the body or when the injection is given deeper than it should be (for example, into the muscle).

If you are worried about the reaction you are having, call your healthcare provider to help you decide if you need medical care. **If the injection site reaction you are having is severe, call your healthcare provider right away.** If you have an injection site reaction, you can discuss with your healthcare provider ways to help the symptoms.

An injection site can get infected. It is important to follow the FUZEON *Injection Instructions* that come with your medicine to lower your chances of getting an injection site infection. **Call your healthcare provider right away if there are signs of infection at the injection site such as oozing, increasing heat, swelling, redness or pain.**

Pneumonia

Patients with HIV get bacterial pneumonia more often than patients without HIV. In clinical trials, patients taking FUZEON with other HIV medicines got bacterial pneumonia more often than patients not receiving FUZEON. It is unclear if this was related to the use of FUZEON. **You should contact your healthcare provider right away if you have a cough, fever or trouble breathing.** Patients are more likely to get bacterial pneumonia if they had a low number of CD4 cells, increased amount of HIV in the blood, intravenous (injected into the vein) drug use, smoking or had experienced lung disease in the past. It is unclear if pneumonia is related to FUZEON.

Allergic reactions

FUZEON can cause serious allergic reactions. Symptoms of a serious allergic reaction with FUZEON can include:

- trouble breathing
- fever with vomiting and a skin rash
- blood in your urine
- swelling of your feet

Call your healthcare provider right away if you get any of these symptoms.

Other side effects

The following side effects were seen more often in patients using FUZEON with their other anti-HIV medicines than in patients not using FUZEON with their other anti-HIV medicines:

- pain and numbness in feet or legs
- loss of sleep
- depression
- decreased appetite

- weakness or loss of strength
- muscle pain
- constipation
- pancreas problems

These are not all the side effects of FUZEON. The list of side effects with FUZEON is **not** complete at this time because FUZEON is still being studied.

If you have questions about side effects, ask your healthcare provider. **Report any new or continuing symptoms to your healthcare provider.** Your healthcare provider will tell you what to do and may be able to help you with these side effects.

How is FUZEON stored?

FUZEON vials not mixed with sterile water can be stored at room temperature (59° to 86°F). FUZEON should be refrigerated if it cannot be stored at room temperature.

The Sterile Water for Injection (diluent) may be stored at room temperature (59° to 86°F).

After FUZEON has been mixed with the sterile water, the vial can be stored in a refrigerator for up to 24 hours.

Do not use FUZEON or sterile water after the expiration date on the vials. Do not keep FUZEON that is out of date or that you no longer need.

General information about the safe and effective use of FUZEON

Medicines are sometimes prescribed for conditions not mentioned in patient information leaflets. Do not use FUZEON for a condition for which it was not prescribed. Do not give FUZEON to other people, even if they have the same symptoms you have. It may harm them. **Keep FUZEON and all medicines out of the reach of children.**

This leaflet summarizes the most important information about FUZEON. If you would like more information, talk with your healthcare provider or see the section, *"Where can I get more information about FUZEON?"* in this leaflet. You can ask your healthcare provider or pharmacist for information about FUZEON that is written for health professionals.

What are the ingredients in FUZEON?

Active Ingredient: enfuvirtide

Inactive Ingredients: Mannitol, sodium carbonate, sodium hydroxide, and hydrochloric acid.

FUZEON comes packaged as a convenience kit containing the following:

- 60 vials of FUZEON (2 cartons of 30 each)
- 60 vials of Sterile Water for Injection (2 cartons of 30 each)
- syringes for mixing (3 cc)
- syringes for injecting (1 cc)
- alcohol pads

Call your healthcare provider or pharmacist if you need more supplies.

Where can I get more information about FUZEON?

The best source for more information about FUZEON is your healthcare provider. Additional information about FUZEON is located at www.FUZEON.com and 1-877-4 FUZEON (1-877-438-9366).

Changes since the last version of this leaflet

This is the first version of this leaflet and was written in March, 2003. Please check this section when your medicine is refilled for any important new information about FUZEON.

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Rx Only

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FUZEON™ (enfuvirtide) Injection Instructions

1 Before You Begin

This is a step-by-step guide to injecting FUZEON™ (enfuvirtide) that helps remind you about what you learned at your healthcare provider's office. Complete information about FUZEON is included in the box with your medicine. If you have any questions about using FUZEON, call your healthcare provider or the pharmacy that provided your FUZEON. These instructions are for an adult dose of 1 mL/cc of FUZEON. If your prescription is for less than 1 mL/cc, or if the prescription is for a child, your healthcare provider may tell you to use different syringes.

Safety Tips

- Wash your hands well before starting. Once your hands are clean, **do not** touch anything except the medicine, supplies, and the area around the injection site
- **Do not** touch the needle when holding the syringe. If you touch the needle, you will need to start over with a new syringe. If you run out of syringes, contact your pharmacy
- **Do not** touch the tops of the vials once they have been cleaned with an alcohol pad. If you do, clean them again with a new alcohol pad. If you run out of alcohol pads, contact your pharmacy
- Make sure none of the items in your kit have been opened. **Do not** use opened materials
- Never mix FUZEON with tap water. Use only the sterile water provided to mix FUZEON
- Never mix anything or any other medicine in the same syringe as FUZEON
- Inject FUZEON just under the skin (subcutaneous). FUZEON should **never** be given directly into your veins (intravenous) or directly into your muscle (intramuscular)
- There should never be any particles floating in the FUZEON once it is completely mixed with sterile water. If you see any, **do not** use that vial—contact the pharmacy that provided your FUZEON
- Use syringes, vials of FUZEON and vials of sterile water only one time

Disposing of Used Syringes, Needles, and Supplies

- Put all used syringes and needles directly into the sharps container
- **Do not** overfill the sharps container
- Keep the cover on the container and keep it out of the reach of children
- Once the container is full, it is important to safely dispose of it. Never throw the sharps container into the trash. Your healthcare provider or the pharmacy that provided your FUZEON can tell you the right way to dispose of the sharps container

- Used alcohol pads and vials can be thrown into the trash. If you see any blood on an alcohol pad, put it in the sharps container
- If you have any other questions about safely disposing of syringes, needles or supplies, please talk to your healthcare provider or the pharmacy that provided your FUZEON

Having Someone Help You With Injections

Certain injection sites, such as the upper arms, can be hard to use at first. If you need help, ask your partner, a friend or a family member. Anyone who will be helping you should know how to inject FUZEON to lower the chance of getting an accidental needlestick or giving you an infection. They should:

- Meet with your healthcare provider to learn the safe way to give injections
- Read the *Caregiver's Guide to Injecting FUZEON*

2 Injection Sites and NMT Syringe Information

Injection Sites

Changing where you inject FUZEON on your body each time is an important way to lessen how bad your injection site reactions get. For more detailed information about each injection site, see *Your Guide to Taking FUZEON*.

About the NMT Safety Syringe

- There are two different-sized NMT Safety Syringes, a 3-mL (large) syringe and a 1-mL (small) syringe
- NMT Safety Syringes are included with FUZEON because the used needle springs back by itself into the syringe after use, lowering the chance of accidental needlesticks

  Important! When first picking up the NMT Safety Syringe or injecting air or sterile water into vials, **do not** push the plunger past the 0.2-mL/cc mark on the barrel of the 3-mL (large) syringe or past the 0.05-mL/cc mark on the barrel of the 1-mL (small) syringe. This could make the needle spring back into the barrel of the syringe or make it hard to pull the plunger back.
- Your healthcare provider may recommend other types of syringes for use with FUZEON
- Never throw your used syringes into the trash. Put them in the sharps container

3 Getting Started

Gather Supplies

Gather the following supplies for each dose and put them on your FUZEON Preparation Mat or a cleaned surface:

- One vial of FUZEON—at room temperature

- One vial of sterile water
- One 3-mL/cc (large) syringe with a 1-inch needle
- One 1-mL/cc (small) syringe with a 1/2-inch needle
- Alcohol pads
- Sharps container

Mixing Two Doses

- To save time, you can mix both of your daily doses of FUZEON at the same time, but you will need to keep the second vial of mixed FUZEON in the refrigerator. **Do not** store mixed FUZEON in the syringe
- Once sterile water has been added to the FUZEON, the vial can be placed in the refrigerator. The FUZEON will dissolve in time for your next dose
- Before using the dose of refrigerated FUZEON, be sure it is clear and allow it to warm to room temperature
- Mixed FUZEON must be used within 24 hours
- The instructions below are for mixing a single dose. If you want to mix two doses at the same time, be sure to use new alcohol pads, syringes, medicine and sterile water
- Write the date and time on the vial when mixed if you are mixing the dose to be used later

Prepare Supplies

- Open the syringe packages and take the caps off the vials
- Throw the syringe packages and vial caps into the trash

Wash Hands

- Wash your hands well using soap and warm water and dry them with a clean towel
- Once your hands are clean, **do not** touch anything other than the medicine, supplies and the area around the injection site

Clean Vial Tops

- Wipe each vial top with a new alcohol pad and let the tops air-dry
- If you touch the rubber tops after cleaning them, clean them again with a new alcohol pad

4 Mixing FUZEON

Draw Up Sterile Water

- Gently tap the FUZEON vial to loosen the powder
- Using the 3-mL/cc (large) syringe, *slowly* pull the plunger back to get 1.1 mL/cc of air

  Important! To avoid causing the needle to spring back into the barrel of the syringe, **do not** push the plunger past the 0.2-mL/cc mark.
- Before turning the sterile water vial upside down, *slowly* inject the air into the vial—and keep the needle in the vial
- Turn the vial upside down. Make sure the tip of the needle is always below the surface of the water to help keep air bubbles from entering the syringe

  Tip! Gently tap or flick the barrel and push and pull the plunger to remove extra air and bubbles. To be sure you end up with 1.1 mL/cc of sterile water in the syringe, you may need to pull the plunger past the 1.1 mL/cc mark.
- *Slowly* pull the plunger back to get 1.1 mL/cc of sterile water into the syringe
- Carefully remove the needle and syringe from the vial

Inject Sterile Water Into FUZEON

- Insert the syringe with sterile water into the FUZEON vial at an angle
- Inject the sterile water slowly, so that it drips down the side of the vial into the FUZEON powder
- Remove the needle from the vial. Push the plunger all the way down with the tip of your thumb until you hear a snap. *This will make the needle spring back into the syringe*
- Put the used syringe in the sharps container

Gently Mix FUZEON

- Gently tap the FUZEON vial with your fingertip for 10 seconds to start dissolving the powder. Then gently roll the FUZEON vial between your hands to reduce the mixing time. Make sure no FUZEON is stuck to the vial wall. After tapping, it could take up to 45 minutes to dissolve

  Important! **Never shake the FUZEON vial.** Shaking will make the medicine foam and it will take much longer to dissolve.
- Once the powder starts to dissolve, just set it aside and it will completely dissolve

Inspect FUZEON

- When completely mixed, the liquid FUZEON should be clear

Important! Completely dissolved FUZEON should be clear and without foam. If the FUZEON is foamy, allow more time for it to dissolve

- If you see bubbles, gently tap the vial until they disappear
- If you see any particles in the FUZEON once it is completely mixed, **do not** use that vial. Contact the pharmacy that provided it
- Mixed FUZEON must be used right away or stored in the vial in the refrigerator and used within 24 hours. **Do not** store mixed FUZEON in the syringe

5 Giving the Injection

Choose the Injection Site

- Using your FUZEON *Planner* to help you, choose a site **different** from the one you used for your last injection

  Important! With the tips of your fingers, feel for any hard bumps. **Do not** inject in or near bumps or any other types of reactions from past injections. Also, **do not** inject into moles, scars, bruises, your belly button or areas that could be irritated by a belt or waistband.

- Clean the injection site with a new alcohol pad. Start in the center, apply pressure and clean in a circular motion, working outward. Allow the site to air-dry

Draw Up FUZEON

- Clean the FUZEON vial top again, using a new alcohol pad. Allow it to air-dry
- Using the 1-mL/cc (small) syringe, pull back the plunger to get 1 mL/cc of air
- Insert the syringe into the vial of mixed FUZEON
- Before turning the vial upside down, *slowly* inject the air into the FUZEON, and keep the needle in the vial

  Important! To avoid causing the needle to spring back into the barrel of the syringe, **do not** push the plunger past the 0.05-mL/cc mark.

- Gently turn the vial upside down
- Make sure the tip of the needle is always below the surface of the FUZEON to help keep air bubbles from entering the syringe. *Slowly* pull the plunger to get 1 mL/cc of FUZEON

  Tip! Gently tap or flick the barrel and push and pull the plunger to remove extra air and bubbles. To be sure you end up with 1 mL/cc of FUZEON in the syringe, you may need to pull the plunger past the 1-mL/cc mark.

- Carefully remove the needle and syringe from the vial

Inject FUZEON

- Pinch and hold a fold of skin around the injection site
- Pierce the skin at a 45-degree angle. The needle should be inserted 3/4 of the way in

  Tip! Your healthcare provider may teach you to inject in a different way.
- With the tip of your thumb, slowly push the plunger all the way to inject FUZEON. *The needle will pull out of the skin and spring back into the syringe by itself when you are done*

  Tip! **Do not** force the needle deeper into the skin while trying to make the needle spring back into the barrel. If you are having a problem, remove the needle from the skin and right away press the plunger down all the way until the needle springs back into the barrel of the syringe.
- Put the used syringe in the sharps container
- Cover the site with a small bandage if you see any blood or medicine

Safety Information

What are the possible side effects of FUZEON?

Injection site reactions

FUZEON causes injection site reactions. Almost all people get injection site reactions with FUZEON. Reactions are usually mild to moderate, but occasionally may be severe. Reactions on the skin where FUZEON is injected include:

- itching
- swelling
- redness
- pain or tenderness
- hardened skin
- bumps

These reactions generally happen within the first week of FUZEON treatment and usually happen again as you keep using FUZEON. A reaction at one skin injection site usually lasts for less than 7 days.

Injection site reactions may be worse when injections are given again in the same place on the body, or when the injection is given deeper than it should be (for example, into the muscle).

If you are worried about the reaction you are having, call your healthcare provider to help you decide if you need medical care. **If the injection site reaction you are having is severe, call your healthcare provider right away.** If you have an injection site reaction, you can discuss with your healthcare provider ways to help the symptoms.

An injection site can get infected. It is important to follow these FUZEON *Injection Instructions* to lower your chances of getting an injection site infection. **Call your healthcare provider right away if there are signs of infection at the injection site such as oozing, increasing heat, swelling, redness or pain.**

Pneumonia

Patients with HIV get bacterial pneumonia more often than patients without HIV. In clinical trials, patients taking FUZEON with other HIV medicines got bacterial pneumonia more often than patients not receiving FUZEON. It is unclear if this was related to the use of FUZEON. **You should contact your healthcare provider right away if you have a cough, fever or trouble breathing.** Patients are more likely to get bacterial pneumonia if they had a low number of CD4 cells, increased amount of HIV in the blood, intravenous (injected into the vein) drug use, smoking or had experienced lung disease in the past. It is unclear if pneumonia is related to FUZEON.

Allergic reactions

FUZEON can cause serious allergic reactions. Symptoms of a serious allergic reaction with FUZEON can include:

- trouble breathing
- fever with vomiting and a skin rash
- blood in your urine
- swelling of your feet

Call your healthcare provider right away if you get any of these symptoms.

Other side effects

The following side effects were seen more often in patients using FUZEON with their other anti-HIV medicines than in patients not using FUZEON with their other anti-HIV medicines:

- pain and numbness in feet or legs
- loss of sleep
- depression
- decreased appetite
- weakness or loss of strength
- muscle pain

- constipation
- pancreas problems

These are not all the side effects of FUZEON. The list of side effects with FUZEON is **not** complete at this time because FUZEON is still being studied. FUZEON is still being studied in children. The safety of FUZEON in children under 6 years of age is not known. The side effects of FUZEON for HIV-positive children aged 6 through 16 years were the same as seen in adult patients.

If you have questions about side effects, ask your healthcare provider. **Report any new or worsening symptoms to your healthcare provider.** Your healthcare provider will tell you what to do and may be able to help you with these side effects.

For more information on FUZEON, please see the patient package insert, www.FUZEON.com, and 1-877-4 FUZEON (1-877-438-9366).

R_X only

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